Examination of Tissue Composition with Attenuated Total Reflection Infrared Microspectroscopy

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A standard method for preserving bone tissue samples is to embed them in paraffin or plastic (methyl methacrylate/dibutyl phthalate). Then they can be sectioned at 5-10 μm thicknesses for transmission infrared experiments. These thin sections are required due to the extremely large signals provided by the phosphate and protein contributions to the infrared spectra. Although embedding provides an excellent means for examining the mineral components of bone, this technique has a few drawbacks including (1) interference of the infrared absorbance of the embedding medium, and (2) it is uncertain whether the embedding process affects protein secondary structure. Conventional freeze/microtome cleavage methods can be used to provide “native” tissue samples. However, it is difficult to section to less than 20 μm, which is problematic due to the high infrared absorbance at those thicknesses. Thus, we are using attenuated total reflection infrared microspectroscopy to examine such native samples (eg. without plastic). Sections of tissue are frozen and cleaved with a microtome and examined using Attenuated Total Reflectance (ATR). ATR is a surface technique that probes the tissue structure approximately 10 μm into the surface based on the evanescent wavefront of the infrared radiation. Due to this property, the samples are examined “wet”. ATR infrared microspectroscopy is a superior method for examining protein spectra, since no denaturing conditions that may perturb the protein structure are required.